

# Phenolic profiles of nectar and honey of *Quillaja saponaria* Mol. (Quillajaceae) as potential chemical markers

Gloria Montenegro, Javiera Díaz-Forestier, Carolina Fredes and Sharon Rodríguez

Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

## ABSTRACT

*Quillaja saponaria* Mol. (Quillajaceae) is one of the most important melliferous species in Chile, mainly as a source of monofloral honey. Honey made by *A. mellifera* presents biological activity against pathogens and antioxidant capacity associated with the presence of phenolic compounds deriving from the nectar, as a result of bee honey foraging. The aim of this study was to identify and quantify the phenolic compounds from the floral nectar of *Q. saponaria* and the honey made in apiaries in the central zone, and compare the composition of the chromatographic profiles of nectar and honey to known phenolic compounds. The results obtained by HPLC-DAD (high-performance liquid chromatography with diode-array detection) showed a similar profile of phenolic compounds, in which gallic acid, myricetin, rutin, quercetin and naringenin were identified. The phenolic compounds detected could be used as a reference for future studies for determining potential chemical markers of this honey, complementing the present identification of honeys by determining their botanical origin. The identification of bioindicators of the floral origins for honey of this species could provide added value to honey commercialization by certifying the botanical origin of their chemical features and biological attributes.

**Key words:** *Apis mellifera*; chromatographic profile; floral nectar; honey; phenolic compounds; *Quillaja saponaria*.

## INTRODUCTION

The composition of floral nectar, especially the relative proportion of sugars and amino acids, plays an important role in plant-pollinator interaction and its evolution (Simpson and Neff, 1983; Real and Rathcke 1988). Nectar is an aqueous plant secretion whose content is mainly sugars and amino acids (Baker and Baker, 1983; Nicolson and Thornburg, 2007; Gonzalez-Teuber and Heil, 2009). Nectar is made by a large number of higher plants in order to attract insect or vertebrate pollinators which transport pollen from plant to plant, becoming a key attribute for increasing cross-pollination as a response to visiting insects (Petanidou, 2005; Pacini and Nepi, 2007). Flowers secreting more nectar show more successful pollination events (Mitchell and Waser, 1992; Nepi *et al.*, 2001; Langenberger and Davis 2002; Maćukanović *et al.*, 2004; Fischer and Leal, 2006; Higginson *et al.*, 2006).

Nectar collected by bees, particularly *Apis mellifera* L., is converted into honey by enzymatic action and dehydration, producing about 18% water content (Moguel *et al.*, 2005). Honey is a sugar-saturated solution and one of the most complex natural carbohydrate mixtures. It contains glucose, fructose and other carbohydrates, which are a combination of at least 11 disaccharides, 11 trisaccharides and several oligosaccharides (Siddiqui and Furgala, 1967, 1968; Low and Sporns, 1988); it also has antioxidants, terpenoids and secondary compounds such as phenols and alkaloids, among others (Nicolson and Thornburg, 2007).

Sugars are the main component of honey (around 95% of the dry weight), with a relative amount of fructose and glucose (60-80%) which is useful for monofloral honey classification (Persano *et al.*, 1995; Piccirillo *et al.*, 1998; Persano and Piro, 2004; Bogdanov *et al.*, 2004). In Chile, Montenegro *et al.* (2004)

identified eight species of native plants showing an intensive use as nectar source by *A. mellifera* and only five species used as the source for monofloral honeys, which are selected by bees mainly due to the volume and chemical composition of nectar offered by the flowers. *Q. saponaria* Mol. (soapbark) is among these native plants; it is an endemic Quillajaceae of Central Chile with highly attractive flowers for honeybees (Ramírez and Montenegro, 2004; Díaz, 2007). Nectar foraging is high and high levels of monofloral honey production are reached (Montenegro *et al.*, 2008). It is highly appreciated in national and international markets, due to its nematocidal and antifungal properties (San Martín and Briones, 1999; Montenegro *et al.*, 2001; Montenegro *et al.*, 2009) and for its known chemical features and biological attributes (Montenegro *et al.*, 2010a, b).

The chemical analysis of nectar samples has been focused on the concentration and relative proportions of carbohydrates (Gil *et al.*, 1995; Gardener and Gillman, 2001). Secondary metabolites, generally appearing in low levels but clearly involved in ecological interactions, have been also reported (Radhika *et al.*, 2010). Extensive research has explained the role played by the components in the antioxidant ability of honey (Busserolles *et al.*, 2002; Gheldof and Engeseth, 2002; Gheldof *et al.*, 2002; Schramm *et al.*, 2003; Muñoz *et al.*, 2007). In addition to polyphenols, honey presents a variety of natural compounds, such as nitrogen compounds, carotenoids and vitamin C, which are widely known for their antioxidant activity (Larson, 1988).

This study assumes that the nectar content of phenolic compounds represents an essential element which, in most cases, defines the level of antioxidant activity of a specific honey sample, although all components act altogether to produce this activity. The aims of this study were to identify

\*Corresponding author: Gloria Montenegro, Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, Santiago, Chile. Fax: (562) 3546726. E-mail: gmonten@uc.cl

and determine quantitatively the phenolic components of floral nectar from *Q. saponaria* and honey produced in apiaries in the central zone of Chile, determine the presence and amount of phenolic compounds in soapbark nectar, and compare the composition of the chromatographic profiles of nectar and honey to known phenolic compounds.

## MATERIALS AND METHODS

### Floral nectar collection

Nectar from flowers of three *Q. saponaria* trees was collected in Manquehue Hill in Santiago (33°22' SL, 70°35' WL, Metropolitan Region). The nectar collected from several flowers of the same tree (experimental unit) was placed in Eppendorf tubes and stored at -20 °C before further analysis in the laboratory. Additionally, nectar from the honey crop of *A. mellifera* foraging on soapbark trees was also extracted. The botanical origin of the nectar was confirmed by microscopic analysis of the existing pollen (Loveaux *et al.*, 1970; Low *et al.*, 1988). The samples were filtered and stored at -20 °C before their analysis.

### Honey collection

The honeys were obtained from apiaries located in the Metropolitan Region and the Region of Maule, Chile, and encoded as RM-012005-M337, VII-032005-M338 and VII-032003-M243, respectively. The botanical origin of the honeys was determined according to Chilean Standard 2981 (INN, 2006). 10 g of honey were diluted in 10 mL of distilled water, and then centrifuged at 2,500 rpm for 5 minutes. The supernatant was eliminated and the sedimented pollen was re-suspended in 0.1 mL of distilled water. Five preparations from each honey sample were elaborated for optical microscopy and the pollen grains from each sample were identified using palinoteque and reference bibliography (Heusser, 1971; Hodges, 1984; Ertmann, 1986; Montenegro, 2000).

### Sample preparation

The nectar from the trees was diluted to 50% with methanol and then centrifuged. Phenolic extracts of the honey and nectar from the bee crops were obtained, following the methodology of Tomás-Barberán *et al.* (1993, 2001).

A volume of 3 mL of nectar was diluted with pH 2 water to a final volume of 12 mL for the elaboration of the nectar extract. The solution was transferred to a small resin column Amberlita XAD-2 (90 mm height x 10 mm diameter). Then it was eluted with 6 mL of pH 2 water, with 12 mL of distilled water, and finally with 18 mL of pure methanol. The methanolic fraction was evaporated to dryness in a stove at 45 °C. The residue was re-suspended in 1 mL of distilled water, and 1 mL of ethyl ether was added. The organic phase was extracted and the process was repeated twice. The resulting solution was concentrated to dryness in a stove at 45 °C, and the residue was re-suspended in 0.12 mL of HPLC-degree-methanol.

### Determination of phenolic compounds

0.02 mL of the extracts and nectar diluted in methanol were injected into a HPLC (HP1100) with a C-18 Agilent reversed-

phase column, with diode-array detection to determine the phenolic compounds from the samples. Two solvents were used for the mobile phase: water and acetonitrile (pH3). A system of elution gradient was used (Markham *et al.*, 1970) (Table 1).

**TABLE 1**  
Gradient of solvents elution mobile phase (solvent A: water; solvent B: acetonitrile (pH 3))

Time (min)	% solvent A	% solvent B
0	84	16
3	84	16
12	40	60
14	40	60
14	84	16

A flow rate of 1 mL minute<sup>-1</sup> was used, with 15 minute elution time. The detection was made at 280 nm and 314 nm. Calibration curves with pure standards in methanol were made in order to identify the phenolic compounds existing in the nectar samples and the extracts. The standards were commercially obtained (Sigma-Aldrich).

### Statistical analysis

The statistical software Infostat was used for the analyses (version 2012, Córdoba, Argentina) using analysis of variance, in order to determine the existence of significant differences among honeys analyzed for each quantified compound. The differences among honeys were determined by the Tukey test ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

Table 2 shows the results from the palynological analysis of the studied honeys. The three honey samples analyzed in this study were by definition monofloral from *Q. saponaria*, as they had more than 45% pollen from this species (INN, 2006), but with different relative percentages of participation. Honey RM-012005-M337 and honey VII-032003-M243 also presented important amounts of *Lithrea caustica*, a typical species from the shrub and sclerophyllous forest of Central Chile. Honey VII-032005-M338 showed the smallest proportion of *Q. saponaria* within the pollen fraction, presenting high percentages of *Mellilotus indicus* and *Lotus uliginosus*, species introduced and ruderal in Chile.

The chromatograms recorded at different wave lengths with HPLC – DAD show that most compounds were detected at 314 nm. The results obtained allowed identification of a series of phenolic compounds; at least 10 have been identified, with gallic acid as the principal compound. The corroboration of the presence of this compound was possible by the addition of growing concentrations of pure standard gallic acid to the samples of nectar extracts.

Fig. 1 presents nectar chromatograms collected from *Q. saponaria* flowers from the studied trees. Fig. 2 shows the chromatograms of the nectar extracts from bees foraging in



soapbark flowers, and Fig. 3 shows the extract chromatograms from the studied honeys.

The analyses by HPLC-DAD of the nectar extracts (of *Q. saponaria* flowers and the crop of honeybees) (Fig. 1 and 2) and the honey extracts (Fig. 3), showed a similar profile of phenolic compounds, which supports the idea that the antioxidant activity of honey may be obtained from the nectar of this endemic shrub of Central Chile.

The main compound identified was *gallic acid*, which corresponds to a phenolic acid whose antioxidant activity equivalent in trolox is 3.01 mmol L<sup>-1</sup> (Miller and Rice-Evans, 1995), with known antimutagenic activity and protective action against liver damage caused by carbon tetrachloride (Urquiaga and Leighton, 2000).

Abscisic acid (ABA) was another compound recorded in all the chromatograms, which is a plant hormone associated with the protection of plants in conditions of environmental

stress. The presence of this hormone in nectar is well known (Goodwin and Mercer, 1983; Ferreres *et al.*, 1996), and therefore it may be present in honey in variable amounts.

Nectar composition is influenced by the environmental conditions of the place where the plant grew (Pacini *et al.*, 2003), which may act on the regular presence of phenolic compounds and abscisic acid, and on some honey compounds. Walters *et al.* (1999) observed that plants may stimulate the synthesis of secondary metabolites, diminishing damages by their ability to capture free radicals under stress conditions, and reduce the penetration of UV-B ultraviolet radiation. These stress conditions increase the ABA concentration in the nectar (Taiz and Zeiger, 1991), and therefore, in honey. The high inter-annual variability of the climate conditions of the central zone of Chile may be associated with the representation of these compounds in the honey produced from *Q. saponaria*.

The results indicated the presence of a predominant phenolic compound, gallic acid, and several similar compounds in the samples of nectar and honey. These compounds could act as chemical markers of floral origin for honeys of this species; however, a larger number of honey samples should be analyzed to determine a correlation among the existing compounds in the nectar and honey from *Q. saponaria*. On the other hand, the effect of other species present in honey, which vary in each production season (Montenegro, 2002; Montenegro *et al.*, 2003, 2005, 2008, 2009, 2010a, 2010b) providing specific phenolic compounds to honey, must be considered.

The phenolic compounds identified in this study are ecologically and productively important: they play an important role in the attraction and nutrition of pollinators (Adler, 2000), they may prevent oxidation of other nectar substances (hinder the entrance of pathogens through the nectary (Cushnie and Lamb, 2005) and might provide an aggregate value to honey commercialization by the certification of the botanical origin of chemical features and biological attributes.

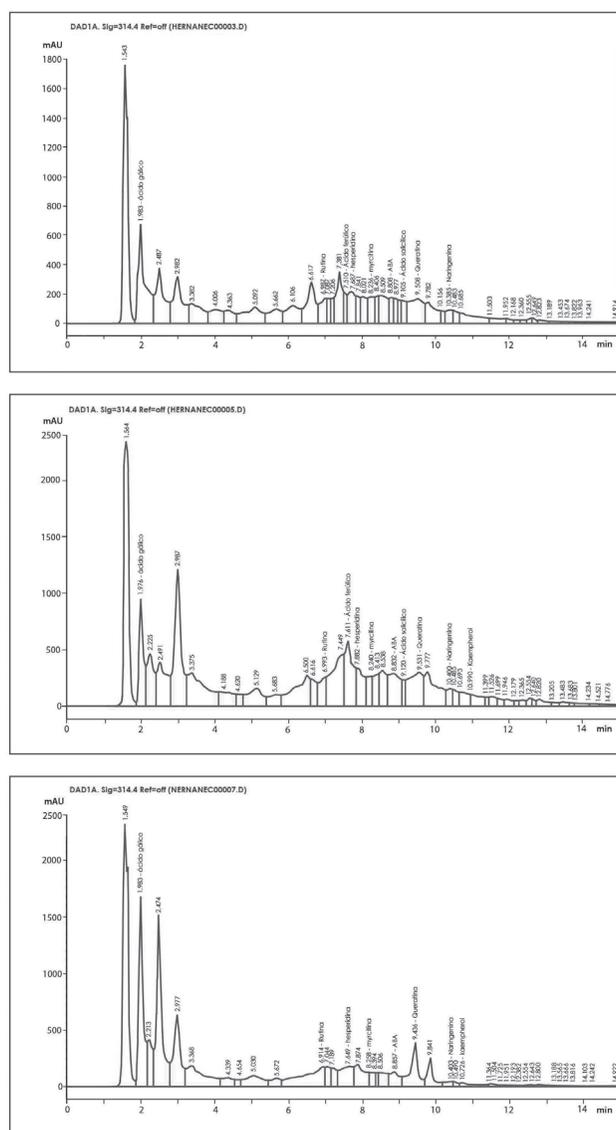
The study concludes that the results on the phenolic composition of the samples of *Q. saponaria* nectar and honeys are the first evidence generated on this topic for an emblematic Chilean species. The establishment of compared chromatographic profiles for nectar and honey from native plant species important for honeybee in Chile will allow the determination of potential chemical honey markers as a supplement to the traditional melisopalynological analysis, which could provide a higher added value to honey by the presence of chemical compounds of high biological activity.

#### ACKNOWLEDGEMENTS

This research was financed by FONDECYT project 1110808 to G. Montenegro.

#### REFERENCES

- ADLER LS 2000. The ecological significance of toxic nectar. *Oikos* 91: 409-420.
- BAKER HG, BAKER I (1983) Floral nectar sugar constituents in relation to pollinator type. In CE Jone, RJ Little [eds.]. *Handbook of experimental pollination biology*, 117 – 141. Van Nostrand Reinhold, New York, New York, USA.
- BOGDANOV S, RUOFF K, PERSANO L (2004) Physico-chemical method for the characterization of unifloral honey: a review. *Apidologie* 35(1): S4–S17.
- BUSSEROLLES J, GUEUX E, ROCK E, MAZUR A, RAYSSIGUIER Y (2002) Substituting honey for refined carbohydrate protects rats from



**Figure 3.** HPLC-DAD chromatograms of phenolic compounds identified in honey extracts (A: RM-012005-337, B: VII-032005-338 and C: VII-032003-M243) obtained at 314 nm.

- hypertriglyceridemic and prooxidative effects of fructosa. *J Agric Food Chem* 50(21): 5870-5877.
- CUSHNIE TP, LAMB AJ (2005) Antimicrobial activity of flavonoids. *Int J Antimicrob Age* 26: 343-356.
- DÍAZ J (2007) Estructura de los nectarios de *Q. saponaria*, dinámica de secreción de néctar y polinizadores asociados: incidencias en la producción de miel. Tesis de Magíster en Recursos Naturales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile.
- ERDTMANN G (1986) Pollen Morphology and Plant Taxonomy; Angiosperms (an Introduction to Palynology). EJ Brill, Leiden, 553 p.
- FERRERES F, ANDRADE P, TOMÁS-BARBERAN FA (1996) Natural occurrence of absicic acid in Portuguese heather honey and floral nectar. *J Agric Food Chem* 44: 2053-2056.
- FISCHER E, LEAL IR (2006) Effect of nectar secretion rate on pollination success of *Passiflora coccinea* (Passifloraceae) in the central amazon. *Braz J Biol* 66: 747-754.
- GARDENER MC, GILLMAN MPJ (2001) Analyzing variability in nectar amino acids: composition is significantly less variable than concentration. *J Chem Ecol* 27: 2545-2558.
- GHELDOF N, ENGESETH NJ (2002) Antioxidant capacity of honey from various floral sources based on the determination of oxygen radical absorbance and inhibition of in vitro lipoprotein oxidation in human serum samples. *J Agric Food Chem* 50(3): 3050-3055.
- GHELDOF N, WANG XH, ENGESETH NJ (2002) Identification of antioxidant components of honeys from various floral sources. *J Agric Food Chem* 50: 5870-5877.
- GIL MI, FERRERES F, ORTIZ R, SUBRA E, TOMÁS-BARBERÁN FAJ (1995) Plant phenolic metabolites and floral origin of rosemary honey. *J Agric Food Chem* 43: 2833-2838.
- GONZALEZ-TEUBER M, HEIL M (2009) Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal Behav* 4: 809-813.
- GOODWIN TW, MERCER EI (1983) Introduction to Plant Biochemistry. ed 2. Pergamon Press, Oxford, England.
- HEUSSER C (1971) Pollen and spores of Chile. The University of Arizona Press. Tucson, Arizona. EE.UU. 167 p.
- HIGGINSON AD, GILBERT FS, BARNARD CJ (2006) Morphological correlates of nectar production used by honeybees. *Ecol Entomol* 31: 269-276.
- HODGES D (1984) The pollen loads of the honeybee, a guide to their identification by colour and form. London Inter Bee Res Assoc, 120 p.
- LANGENBERGER MW, DAVIS AR (2002) Temporal changes in floral nectar production, reabsorption, and composition associated with dichogamy in annual Caraway (*Carum carvi*; Apiaceae). *Am J Botany* 89(10): 1588-1598.
- LARSON RA (1988) The antioxidants of higher plants. *Phytochemistry* 27: 969-978.
- URQUIAGA I, LEIGHTON F (2000) Plant Polyphenol Antioxidants and Oxidative Stress. *Biol Res* 33 (2): 55-64.
- LOVEAUX J, MAURIZIO A, VORWOHL G (1970) Methods of melissopalynology. *Bee World* 51: 125-138.
- LOW NH, SPORNS P (1988) Analysis and Quantitation of Minor Diand Trisaccharides in Honey, Using Capillary Gas Chromatography. *J Food Sci* 53(2): 558-561.
- LOW NH, NELSON DL, SPORNS P (1988) Carbohydrate Analysis of Western Canadian Honeys and their Nectar Sources to Determine the Origin of Honey Oligosaccharides. *J Apic Res* 27(4): 245-251.
- MAČUKANOVIĆ M, DULETIĆ S, JOCIĆ G (2004) Nectar production in three melliferous species of Lamiaceae in natural and experimental conditions. *Acta Veterinaria (Beograd)* 54(5-6): 475-487.
- MARKHAM K, MABRY TJ, THOMAS MB (1970) The systematic identification of flavonoid. Springer Verlag, Berlin-New York. p. 1-175.
- MILLER NJ, RICE-EVANS CA (1995) Antioxidant activity of resveratrol in red wine. *Clin Chem* 41:1789.
- MITCHELL RJ, WASER NM (1992) Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. *Ecology* 73: 633-638.
- MOGUEL Y, ECHAZARRETA C, MORA R (2005) Calidad fisicoquímica de la miel de abeja *A. mellifera* producida en el estado de Yucatán durante diferentes etapas del proceso de producción y tipos de floración. *Técnica Pecuaria en México* 43(3): 323-334.
- MONTENEGRO G (2000) Chile nuestra flora útil, guía de uso apícola, medicinal folclórica, artesanal y ornamental. Ediciones Universidad Católica de Chile. Santiago, Chile. 153 p.
- MONTENEGRO G, PEÑA R, TIMMERMAN BN (2001) La corteza de quillaja (*Quillaja saponaria* Mol.), un recurso de la farmacopea internacional. *Rev Acad Colomb Cienc Exact Fis Nat* 25: 420-427.
- MONTENEGRO G, PIZARRO R, ÁVILA G, GÓMEZ M, DÍAZ J, ORTEGA X GONZÁLEZ L (2004) Determinación de la flora melífera nativa de Chile, mediante el análisis palinológico de la miel. X Congreso Nacional de Botánica del Perú. Universidad Nacional de Trujillo, Trujillo, Perú.
- MONTENEGRO G, GÓMEZ M, DÍAZ J, PIZARRO R (2008) Aplicación de la Norma Chilena Oficial de denominación de origen botánico de la miel para la caracterización de la producción apícola. *Cien Inv Agr* 35(2): 181-190.
- MONTENEGRO G, SALAS F, PENA R, PIZARRO R (2009) Actividad antibacteriana y antifúngica de mieles monoflorales de *Quillaja saponaria*, especie endémica de Chile. *Phyton* 78: 141-146.
- MONTENEGRO G (2002) Chile, nuestra flora útil. Guía de plantas de uso apícola alimentario, medicinal folclórico, artesanal y ornamental. Ed. Universidad Católica de Chile. Santiago, Chile. 267 pp.
- MONTENEGRO G, PIZARRO R, ÁVILA G, CASTRO R, RÍOS C, MUÑOZ O, BAS F, GÓMEZ M (2003) Origen botánico y propiedades químicas de las mieles de la Región Mediterránea Árida de Chile. *Cien Inv Agr* 30(3):161-174.
- MONTENEGRO G, PIZARRO R, ÁVILA G, MUÑOZ O, MUJICA AM BAS F (2005) Determination of the botanical origin and some chemical properties of honeys from the central zone of Chile. *Phyton Sp Iss*: 213-223.
- MONTENEGRO G, GÓMEZ M, DÍAZ-FORESTIER J, PIZARRO R (2008) Aplicación de la Norma Chilena Oficial de denominación de origen botánico de la miel para la caracterización de la producción apícola. *Cien Inv Agr* 35(2): 181-190.
- MONTENEGRO G, SALAS F, PEÑA RC, PIZARRO R (2009) Actividad antibacteriana y antifúngica de mieles monoflorales de *Quillaja saponaria*, especie endémica de Chile. *Phyton* 78: 141-146.
- MONTENEGRO G, RODRÍGUEZ S, VÍO S, GÓMEZ M, PIZARRO P, MUJICA AM, ORTEGA X (2010a) Investigación científica y tecnológica en productos apícolas. Fundación COPEC-Universidad Católica. Santiago, Chile. 240 p.
- MONTENEGRO G, PEÑA RC, PIZARRO R (2010b) Multivariate analysis of pollen frequency of the native species *Escallonia pulverulenta* (Saxifragaceae) in Chilean honeys. *Rev Bras Bot* 33(4): 615-630.
- MUÑOZ O, COPAJA S, SPEISKY H, PEÑA R, MONTENEGRO G (2007) Contenido de flavonoides y compuestos fenólicos de mieles chilenas e índice antioxidante. *Química Nova* 390: 848-851.
- NEPI M, GUARNIERI M, PACINI E (2001) Nectar secretion, reabsorption, and sugar composition in male and female flowers of *Cucurbita pepo*. *Intl J Plant Sci* 162: 353-358.
- NICOLSON SW, THORNBURG RW (2007) Nectar chemistry. In SW Nicolson, Nepi and Pacini (eds.) Nectaries and nectar. Springer, Dordrecht, Netherlands.
- INN-INSTITUTO NACIONAL DE NORMALIZACIÓN (2006) NCh 2981 Of2005: Miel de abejas—Denominación de origen botánico mediante ensayo melisopalínológico. Declarada Norma Chilena Oficial de la República el 14 de diciembre de 2005 por Decreto Exento No 765 del Ministerio de Agricultura, publicada en el *Diario Oficial* No 38.358 el 9 de enero de 2006.
- PACINI E, NEPI M, VESPRINI JL (2003) Nectar biodiversity: a short review. *Plant Syst Evol* 238: 7-21.
- PACINI E, NEPI M (2007) Nectar production and presentation. In SW Nicolson, Nepi and Pacini (eds.) Nectaries and nectar. Springer, Dordrecht, Netherlands.
- PERSANO L, PIAZZA M, SABATINI A, ACCORTI M (1995) Characterization of unifloral honey. *Apidologie* 26(6): 453-465.
- PERSANO L, PIRO R (2004) Main European Unifloral Honey: descriptive sheets. *Apidologie* 35(1): 38-81.
- PETANIDOU T (2005) Sugars in Mediterranean floral nectars: An ecological and evolutionary approach. *J Chem Ecol* 31: 106-1088.
- PICCIRILLO G, RODRÍGUEZ B, OJEDA G (1998) Estudio de algunos parámetros fisicoquímicos en mieles cosechadas durante la época seca de ocho zonas apícolas del Estado Zulia, Venezuela. *Revista de la Facultad de Agronomía Universidad de Zulia (Venezuela)* 15: 486-497.
- RADHIKA V, KOST C, BOLAND W, HEIL M (2010) The Role of Jasmonates in Floral Nectar Secretion. *PLoS ONE* 5(2): e9265.
- RAMÍREZ R AND G MONTENEGRO (2004) Certificación del origen botánico de miel y polen corbicular pertenecientes a la comuna de Litueche, VI Región de Chile. *Cien Inv Agr* 31(3): 197-211.
- REAL L, RATHCKE BJ (1988) Patterns of individual variability in floral resources. *Ecology* 69(3): 728-735.
- SAN MARTÍN R, BRIONES R (1999) Industrial uses and sustainable supply of *Quillaja saponaria* saponins. *Econ Botany* 53: 302- 311.

- SCHRAMM DD, KARIM M, SCHRADER HR, HOLT RR, CARDETTI M, KEEN CL (2003) Honey with high levels of antioxidants can provide protection to healthy human subjects. *J Agric Food Chem* 51(5): 1500-1505.
- SIDDIQUI IR, FURGALA B (1967) Isolation and Characterization of Oligosaccharides from Honey. Part I. Disaccharides. *J Apic Res* 6(3): 139-145.
- SIDDIQUI IR, FURGALA B (1968) Isolation and Characterization of Oligosaccharides from Honey. Part II. Trisaccharides. *J Apic Res* 7(1): 51-59.
- SIMPSON BB, NEFF JL (1983) Evolution and diversity of floral rewards. *In* Handbook of experimental pollination biology. JONES CE, LITTLE RJ (eds.). New York: Van Nostrand Reinhold Co. p. 142-159.
- TAIZ L, ZEIGER E (1991) *Plant Physiology*. The Benjamin/Cummings Publishing Company, Inc., Redwood City. 565 p.
- TOMÁS-BARBERÁN FA, FERRERES F, BLÁZQUEZ MA, GARCÍA-VIGUERA C, TOM-LORENTE F (1993) High-performance liquid chromatography of honey Flavonoids. *J Chromat* 634: 41-46.
- WALTERS R, ROGERS J, SHEPARD F, HORTON F. 1999. Acclimation of *ArabidopsisThaliana* to the light environment: the role of photoreceptors. *Planta* 209: 517-527.